# The Role of the Oscillating Min Proteins in the *Escherichia coli* Cell Cycle

Research Thesis

Presented in partial fulfillment of the requirements for graduation *with research distinction* in the undergraduate colleges of The Ohio State University

by

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# Acknowledgements

My sincere gratitude goes to Dr. Adriana Dawes for encouraging me to take part in her molecular genetics lab at The Ohio State University for the past two years. Her undoubtedly positive impact on my career as a budding scientist will be forever appreciated. Thank you to my partner, Natalie Hurst, who helped me to advance further in this project than I may have on my own. You have become a dear friend through this. Thank you to the members of the Dawes Lab for your continuous support, kind and constructive words, paper reviews, and the many teaching moments we have shared. Thank you to Dr. Helen Chamberlin for reviewing my paper and being a committee member for my thesis.

## Abstract

The bacterium *Escherichia coli* expresses the Min proteins from a single operon. MinC, MinD, and MinE make up an oscillatory system that sets up the mid plane division site by discouraging off center division. Post cytokinesis, most cells display a plateau oscillating pattern, a spatially and temporally unordered pattern, until an unknown cue triggers the transition of the oscillations to a spatially and temporally periodic pattern, sawtooth. Here I describe analysis done on the two major time points in the *E.coli* timeline: the time of transition between oscillation patterns and the time of cell division. We induced Min-GFP over-expression with IPTG and tested different aspects of the cell cycle, aiming to explain the role the Min proteins have in these events. There is evidence for an unknown source regulating characteristics of the cell cycle, and we show three different things that could not be the regulator. We found that the pole to pole length at transition fits a normal distribution curve, as well as the pole to pole length at division, meaning length is not absolute and therefore is not a strong dictator in these events. Our results show that time is not directing division, and division could happen at any time following transition. Lastly, it has been widely thought that protein abundance correlates linearly with the length of an *E.coli* cell, but our results shows that in the strains tested there is no significant relationship, showing that proteins abundance is not in control.

# Introduction

*Escherichia coli* is a large and diverse rod shaped bacteria group that are facultatively anaerobic and Gram-negative. *E. coli* utilizes FtsZ, a homolog for tubulin, to assist in cytokinesis by polymerizing into protofilaments that arrange into a ring-like structure, called the Z ring, in the center of the cell (Rowlett et al., 2013). Cytokinesis is regulated by the Min system, the Min proteins, MinC, MinD, and MinE are responsible for setting up the division plane within a parent cell by preventing aberrant division from FtsZ ring assembly at positions other than mid-cell (Shih, 2003). The Min proteins discourage off-center cell wall invagination by oscillating from pole to pole, which ensures proper cell division by preventing organization of the Z ring in areas of high concentrations of Min proteins (Bisicchia, 2013).

MinC prohibits the polymerization of off-center FtsZ rings, MinD is an ATPase that recruits both MinE (in small amounts) and MinC to form complexes on the cytoplasmic side of the membrane, and the vast majority of MinE forms a ring around the outer edge of the MinC/ MinD complexes (called an MinE ring) to avert them from mid-cell association and dissociates MinC from MinD to release the complexes (Meinhardt et al., 2001; Loose et al., 2011). The oscillations occur because MinD attaches to one end of the cell at a time and then recruits MinC and MinE and once the complex is detached the proteins diffuse to the opposite pole in a wave-like fashion. Therefore, oscillations are generated from the proteins cycling through states of collective binding and unbinding to the membrane (Loose, et al. 2010)(Figure 1). In the absence of MinC, asymmetrical divisions near the poles and the formation of mini cells is observed. In the absence of MinE, an even distribution of MinD occurs, preventing cytokinesis, resulting in abnormally long cells. When MinD is absent, MinC and MinE remain in the cytoplasm and are not recruited to the membrane, therefore no oscillations are possible (De Boer et al., 1992).

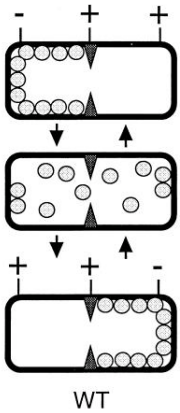



***Figure 1**. A schematic of MinD oscillations from pole to pole while the MinE ring (triangles) remains in the center in a wild type* E.coli*. The '-' indicates areas that are blocked by MinC/MinD complexes, the '+' indicates areas not blocked by the MinC/MinD complexes. The proteins alternate from one side of the MinE ring to the other to block off center cell division (Raskin et al., 1999).*

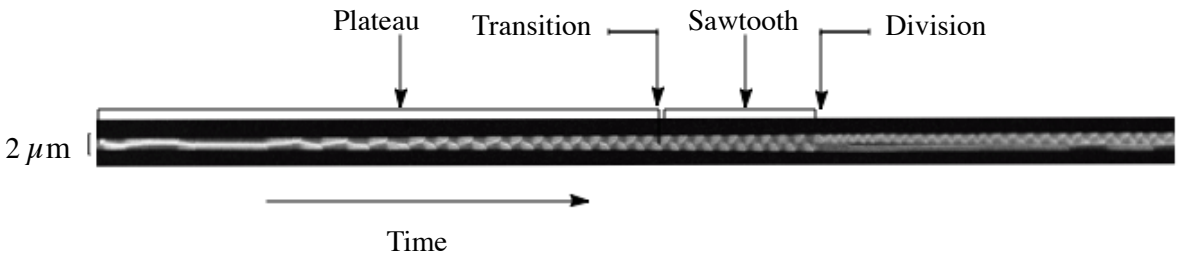



***Figure2**. A kymograph, compilation of all time frames stacked next to each other to see a moving pattern, shows an example of plateau oscillations, a transition to sawtooth pattern, then division into daughter cells. Images are of the strain Plac-MinD Plac-MinE-GFP.*

The two types of oscillation are: plateau, a random spatiotemporal movement in which the proteins have no standard for time allotted at each pole, and sawtooth, a very structured repeated movement in which the proteins spend equal time at each pole (Figure 2). There are two major chronological events in the *E.coli* cell cycle that are of interest in this study; The first event is the transition from plateau oscillations to sawtooth oscillations by an unknown cue and the second event is cell division via binary fission.

Several questions arise pertaining to the events in the *E.coli* cell cycle and previous studies have worked to uncover the dynamics of the self regulating Min proteins. A study has found that there is a critical length in which E. coli cells all display the same oscillation pattern (Fischer-Friedrich et al., 2010). This indicates that length is a good predictor of the type of oscillations occurring in the strains they tested. Separate experiments have shown in various ways that protein abundance correlates with the length of an *E.coli* cell (Milo, 2013; Dennis, et al. 1974). These studies show regulation of the *E. coli* cell and our experiment aimed at answering what role the Min proteins play in dictating the transition of protein oscillation patterns and cell division. We find that there is strong evidence for regulation on various levels, but show many aspects of the cell that are not significantly related, and thus not the regulator of

the cell cycle. Our study shows that in strains with over expressed MinD and MinE proteins, length at transition and length at division are not strictly regulated, as we find no absolute length a which either event occurs, only a preferred length. Time passed between events plays no significant role in how the events are regulated, and concentration of the Min proteins are not correlated with length of the bacteria. Taniguchi et al. in 2010 has found data that supports our results for lack of a correlation between cell length and protein abundance.

## Materials and Methods

### Strains

All nonpathogenic strains used in this experiment were kindly provided by the de Boer Lab at Case Western Reserve University. These bacteria have MinC and MinD deletion backgrounds, though all contain an extra plasmid that express the tagged MIN proteins when induced by Isopropyl β-D-1-thiogalactopyranoside (IPTG). Cultures were grown in the presence of carbenicillin (carb) to ensure all bacteria tested had the proper plasmid insert and the Green Fluorescence Protein (GFP) used is mut2 version.

| Genotype | Protein | | |
|---|---|---|---|
| | MinC | MinD | MinE |
| **Plac-MinD Plac-MinE-GFP** | + | —, Lac | —, Lac-GFP |
| **Plac-MinD-GFP Plac-MinE** | + | —, Lac-GFP | —, Lac |
| **MinC- MinD- Plac-MinE-GFP** | — | — | —, Lac-GFP |
| **Plac-BFP-MinD Plac-MinE-GFP** | + | —, Lac-BFP | —, Lac-GFP |
| **Plac-GFP-MinD Plac-MinE-BFP** | + | —, Lac-GFP | —, Lac-BFP |

***Table 1.*** *A detailed description of all strains used in the experiment and the MIN proteins in each. A '+' means the strain contains the endogenous gene and a '—' indicates that protein is missing in the genome. BFP is Blue Fluorescence Protein. "*Lac*" indicates the* lac *promoter is driving expression of the protein from a plasmid in a genomic mutant background.*

## Growth media

Bacteria were streaked on a plate and grown in the presence of antibiotics for 12-24 hours at 30°C. Individual colonies were harvested for inoculation in 3mL Lysogeny Broth medium (LB) with 20 μg/mL carb in a culture tube with an aerated cap and kept at 30°C in a shaking incubator for 8-12 hours. Cultures were kept in log phase at 30°C by serial inoculations every 8-12 hours. For consistency, each culture was re-inoculated at least once. Four hours prior to imaging, 3mL Low Yeast Lysogeny Broth (LYLB) was inoculated with 15 μL liquid bacteria culture, 20 μg/mL carb, and 20 μg/mL IPTG. LYLB minimizes background noise of the fluorescence when imaging. The culture was placed in a 30°C shaking incubator for 3-4 hours until a desired Optical Density (OD) of 0.2-0.4 absorbance (A) to ensure log phase growth, and then was used for further analysis. Once the sample grows above 0.5 A, the *E.coli* have become too overcrowded and are unusable for an accurate analysis, as they are then in the stationary phase and the growth is limited by exhaustion of available nutrients and space (Todar, 2012). The cultures were concentrated by a factor of two and the bacterial pellet was resuspended in the remaining supernatant for imaging.

**Imaging**

A sample of 0.2 µL resuspended *E. coli* was placed on a cover slip and inverted on a bed of gelatin made from 0.9 mL M9 Egg Salts (118 mM NaCl, 40 mM KCL, 3.4 mM CaCl2, 3.4 mM MgCl2), and 0.3 g powdered porcine gelatin on a microscope slide. The coverslip was sealed to the microscope slide using Petroleum Jelly. The system used for imaging was a Zeiss LSM700 scanning confocal microscope that was attached to an Axio Observer.Z1 inverted microscope, ZEN Black software from Zeiss was used for acquisition, and a 488 nm solid state diode laser. Data points were recorded every 3 seconds for two hours, and a Zeiss Definite Focus focal drift correction system was used to keep the bacteria in the field of view. The BFP signal under the microscope was not sufficiently strong to be able to distinguish from background noise, therefore no analysis was done using BFP signals. All fluorescence referred to is GFP and the amount of fluorescence directly correlates to the amount of tagged MIN proteins in the cell (Wu et al., 2005), therefore a measure of fluorescence is a measure of the MIN protein abundance. The GFP gain and TPMT gain were consistently set to 600 and 300, respectively, with a pinhole size opened up to 1.75 Airy Units to capture the fluorophores being illuminated through the entire three dimensional *E. coli*, a bit depth of 16, and a frame size of x: 692 y:439. Kymographs were made and analyzed using FIJI image analysis software. Graphs were made using Windows Numbers and Gaussian curve fittings were calculated using MATLAB software.

## Results

Fluorescence for all figures refers to the total fluorescence in a region of interest, as opposed to average fluorescence. The strain 'MinC- MinD- Plac-minE-GFP' was only used in the analysis for Figure 3, because it had no oscillations at all. The 'MinC- MinD- Plac-MinE-GFP' genome entirely lacks MinD, which is responsible for drafting MinC to the membrane for the oscillations. The strain 'Plac-MinD-GFP Plac-MinE' was not used in any analysis involving a transition event, as it exhibited\ strictly a sawtooth pattern for every single cell analyzed.

**Figure 3. Correlation Between Cell Length at Transition and Cell Length at Division**

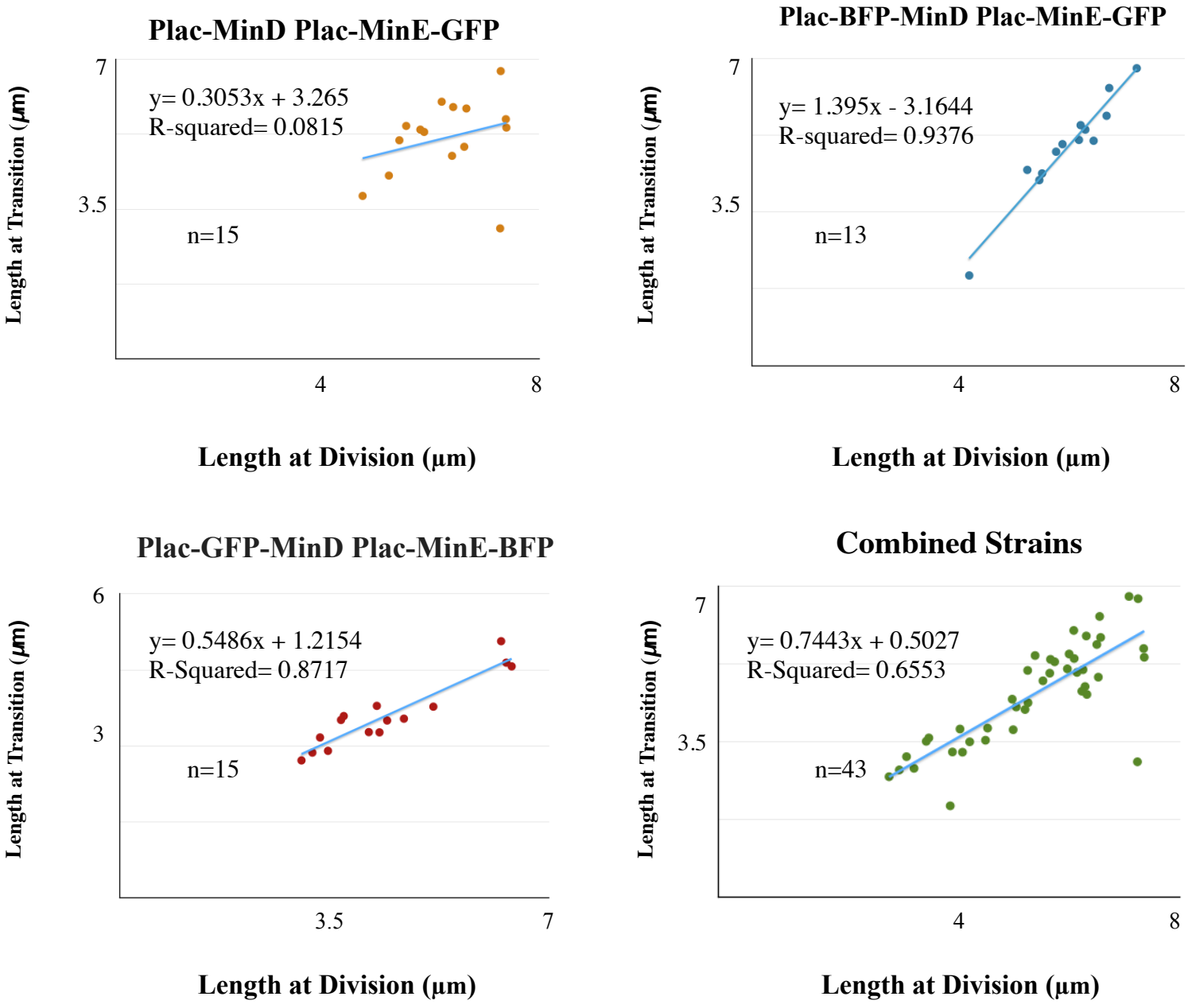





*Figure 3. Significant correlation between cell length at transition and cell length at division for strains Plac-BFP-MinD Plac-MinE-GFP and Plac-GFP-MinD Plac-MinE-BFP. When the data from all three strains tested were combined, there is a significant correlation showing that this property is conserved among strains.*

## Figure 4. Correlation Between Fluorescence at Transition and Fluorescence at Division

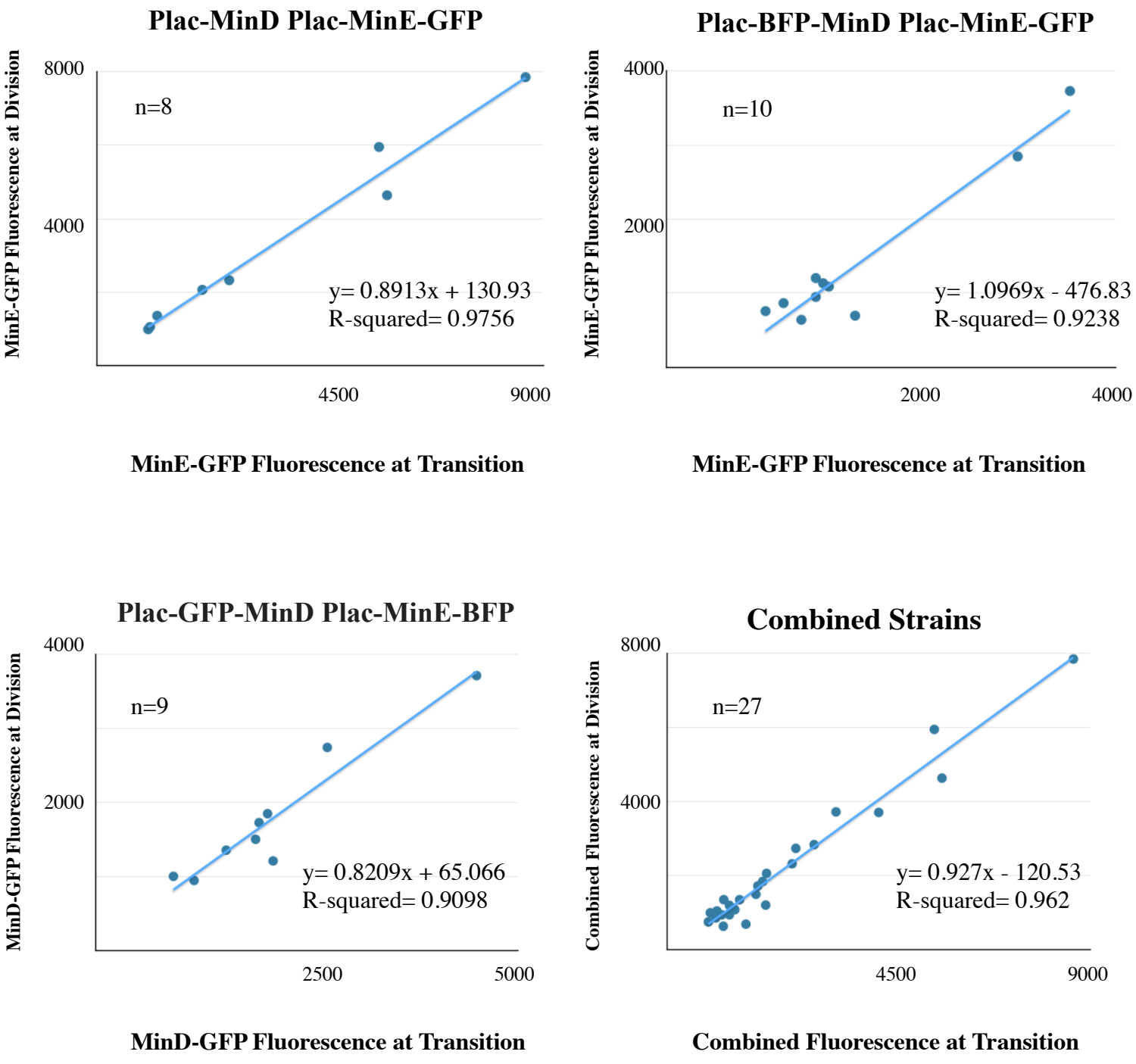



*Figure 4. There is a significant correlation between the relative abundance of Min proteins at transition and at division in all three strains tested. When the three strains are combined, the significant correlation persists which indicates this property is not strain dependent. There is strict regulation of the concentration of Min proteins in each event.*

## Figure 5. Variability in Protein Movement Decreases into Transition

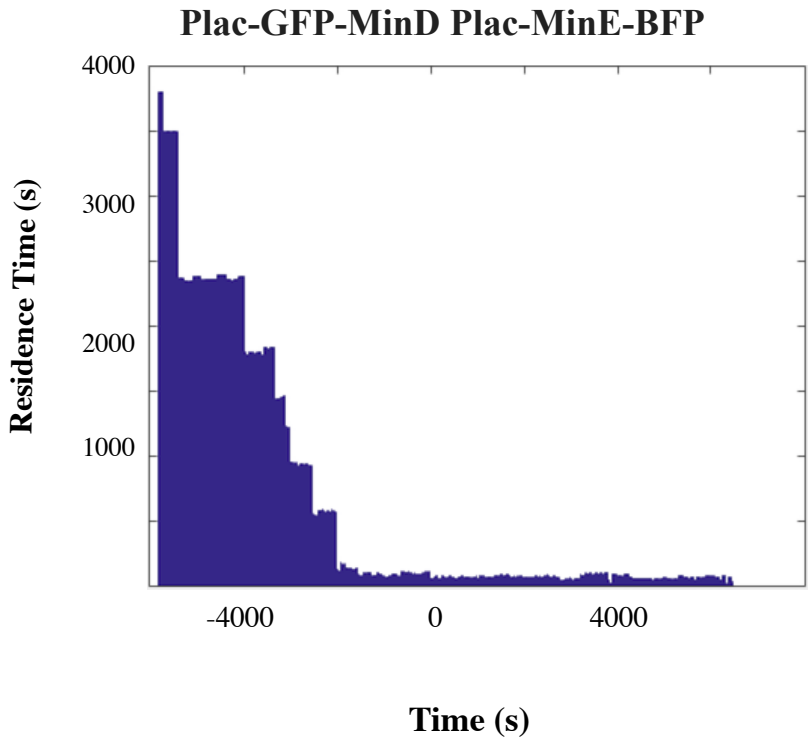
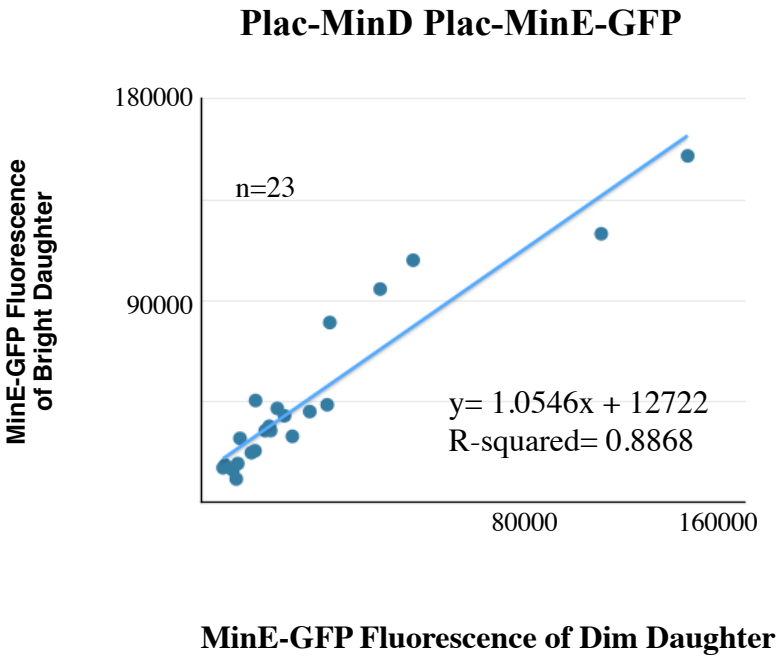
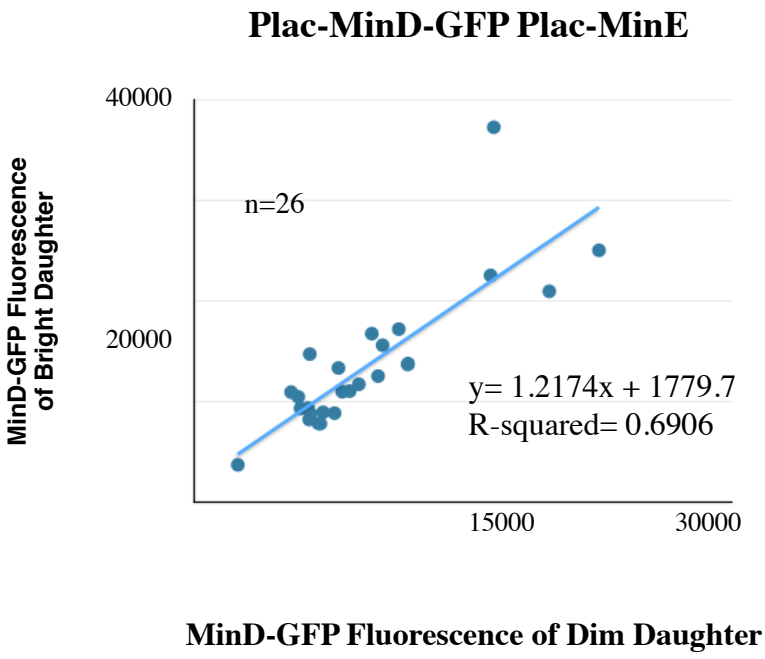
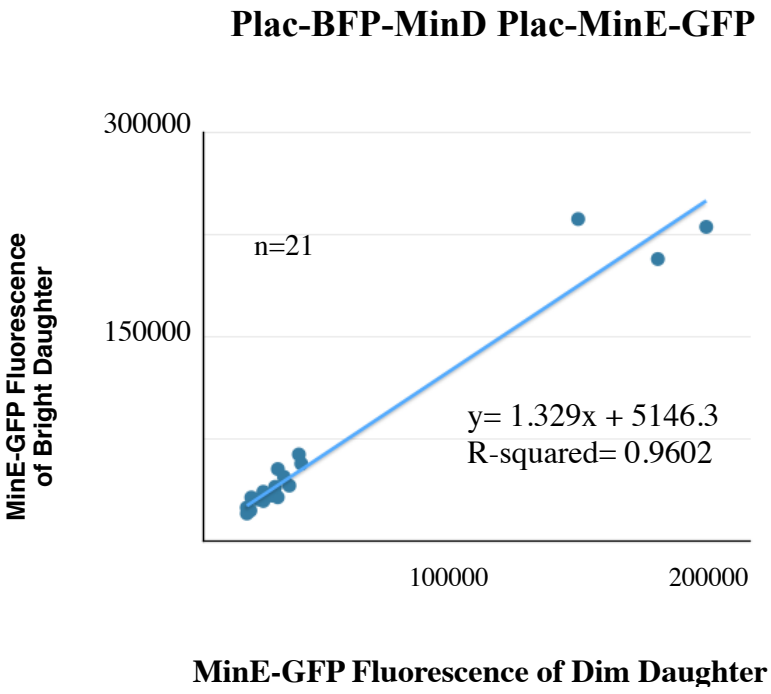


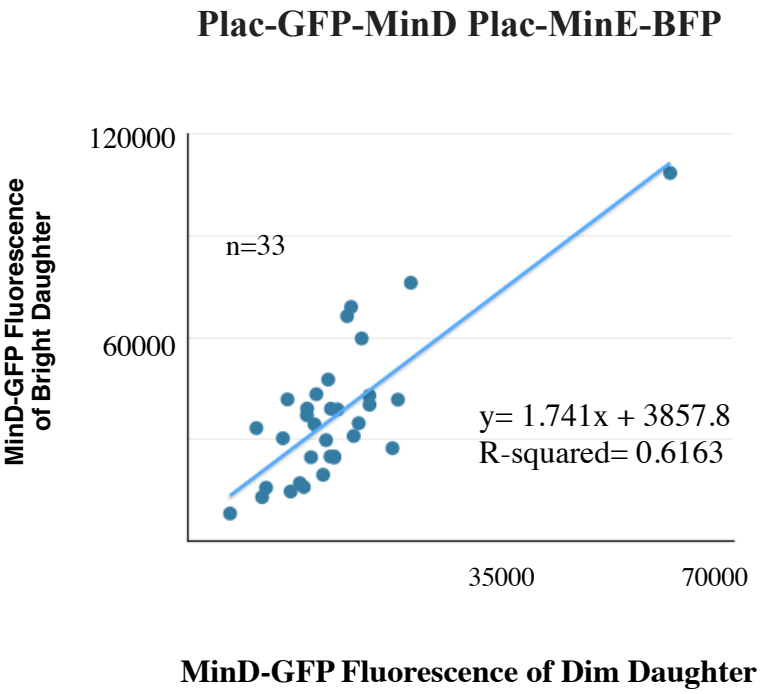

*Figure 5. This figure is calculated by splitting a time series in half horizontally and measuring the fluorescence in that half. The 0 indicates the point of transition within this strain, plateau oscillations occur before the 0, and sawtooth oscillations occur after the 0. The time that the Min proteins spend at each pole decreases along with the variability in oscillations as the cell enters transition between oscillation patterns.*

**Figure 6. Significant Correlation in Asymmetrical Min Inheritance at Division**



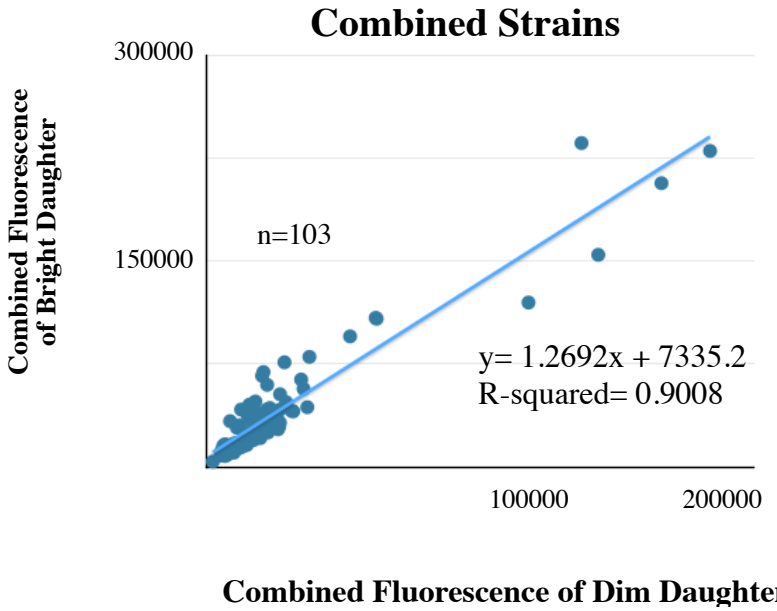



*Figure 6. The distribution of Min proteins is asymmetrical between the two daughter cells, resulting in one daughter being distinctly brighter in fluorescence than the other. The amount of fluorescence in each daughter is significantly correlated. When the four strains tested are combined into one cohort, the correlation persists which indicates this property is not strain dependent. The slope of the regression line is close to 1, indicating that a certain amount of proteins get inherited to the bright daughter.*

**Evidence for Regulation**

There is significant correlation between different aspects of the cell cycle, which indicates regulatory processes in the two different events. The reliable and repeatable patterns of correlation shown above are independent of genotype, meaning the mechanism of control is conserved for all strains. The pole to pole length at transition and division are significantly correlated, suggesting there is some kind of a cue dictating the events (Figure 3). Fluorescence at transition and division show a significant relative relationship (Figure 4). Residence time of the Min proteins in each pole decreases along with variability in their oscillations as they move into transition, showing that a source is telling the cell to prepare for this event (Figure 5). Asymmetrical Min protein inheritance upon division is evident among our results, indicating that during each division event, the distribution of proteins is being regulated for each daughter cell (Figure 6).

**Figure 7. Cell Length at Transition Fits a Normal Distribution Curve**

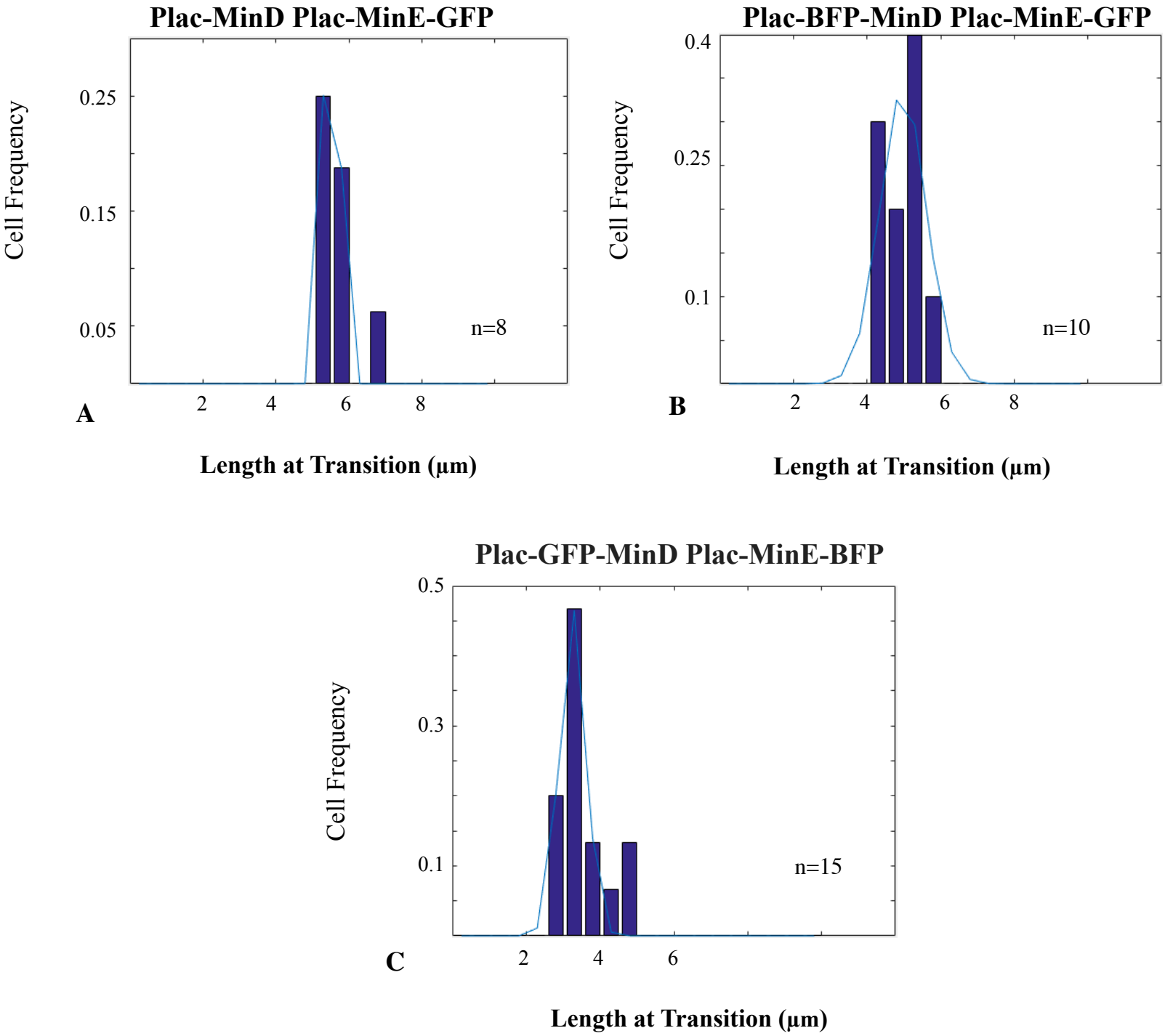



*Figure 7. Length at transition is normally distributed around a mean for all three strains tested. There is a preferred length at transition, but not an absolute length at which the cell transitions between oscillation patterns.*
*Gaussian fit statistics:*

**A.** *The mean is around 5.5 microns. The goodness of fit statistics for this Gaussian Fit are: Sum of Squares due to Error= 0.01563, R-Squared= 0.9561, Adjusted R-Square= 0.951, and Root Mean Squared Error= 0.03032.*

**B.** *The mean is around 5.0 microns. The goodness of fit statistics for this Gaussian Fit are: Sum of Squares due to Error= 0.04543, R-Squared= 0.8183, Adjusted R-Square= 0.7969, and Root Mean Squared Error= 0.05169.*

**C.** *The mean is around 3.5 microns. The goodness of fit statistics for this Gaussian Fit are: Sum of Squares due to Error= 0.02167, R-Squared= 0.9125, Adjusted R-Square= 0.9022, and Root Mean Squared Error= 0.0357.*

**Figure 8. Cell Length at Division Fits a Normal Distribution Curve**

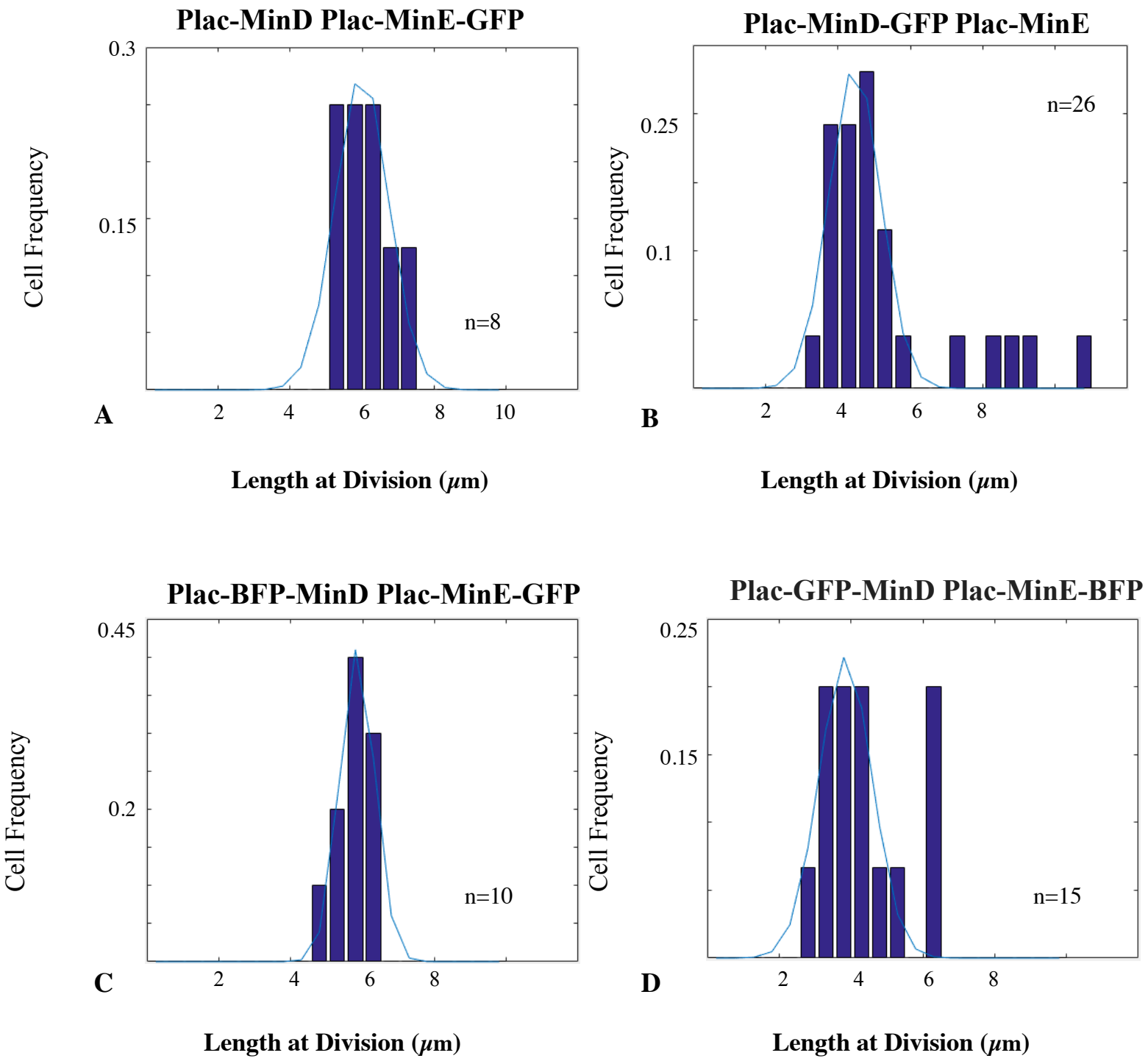



*Figure 8. Length at division is normally distributed around a mean for all four strains tested. There is a preferred length at division, but not an absolute length at which the cell divides.*
*Gaussian fit statistics:*

**A.** *The mean is around 6.5 microns. The goodness of fit statistics for this Gaussian Fit are: Sum of Squares due to Error= 0.01697, R-Squared= 0.8994, Adjusted R-Square= 0.8876, and Root Mean Squared Error= 0.0316.*

**B.** *The mean is around 4.5 microns. The goodness of fit statistics for this Gaussian Fit are: Sum of Squares due to Error= 0.01162, R-Squared= 0.8898, Adjusted R-Square= 0.8782, and Root Mean Squared Error= 0.02473.*

**C.** *The mean is around 5.5 microns. The goodness of fit statistics for this Gaussian Fit are: Sum of Squares due to Error= 0.008745, R-Squared= 0.965, Adjusted R-Square= 0.9609, and Root Mean Squared Error= 0.02268.*

**D.** *The mean is around 4 microns. The goodness of fit statistics for this Gaussian Fit are: Sum of Squares due to Error= 0.04433, R-Squared= 0.6406, Adjusted R-Square= 0.5983, and Root Mean Squared Error= 0.05106.*

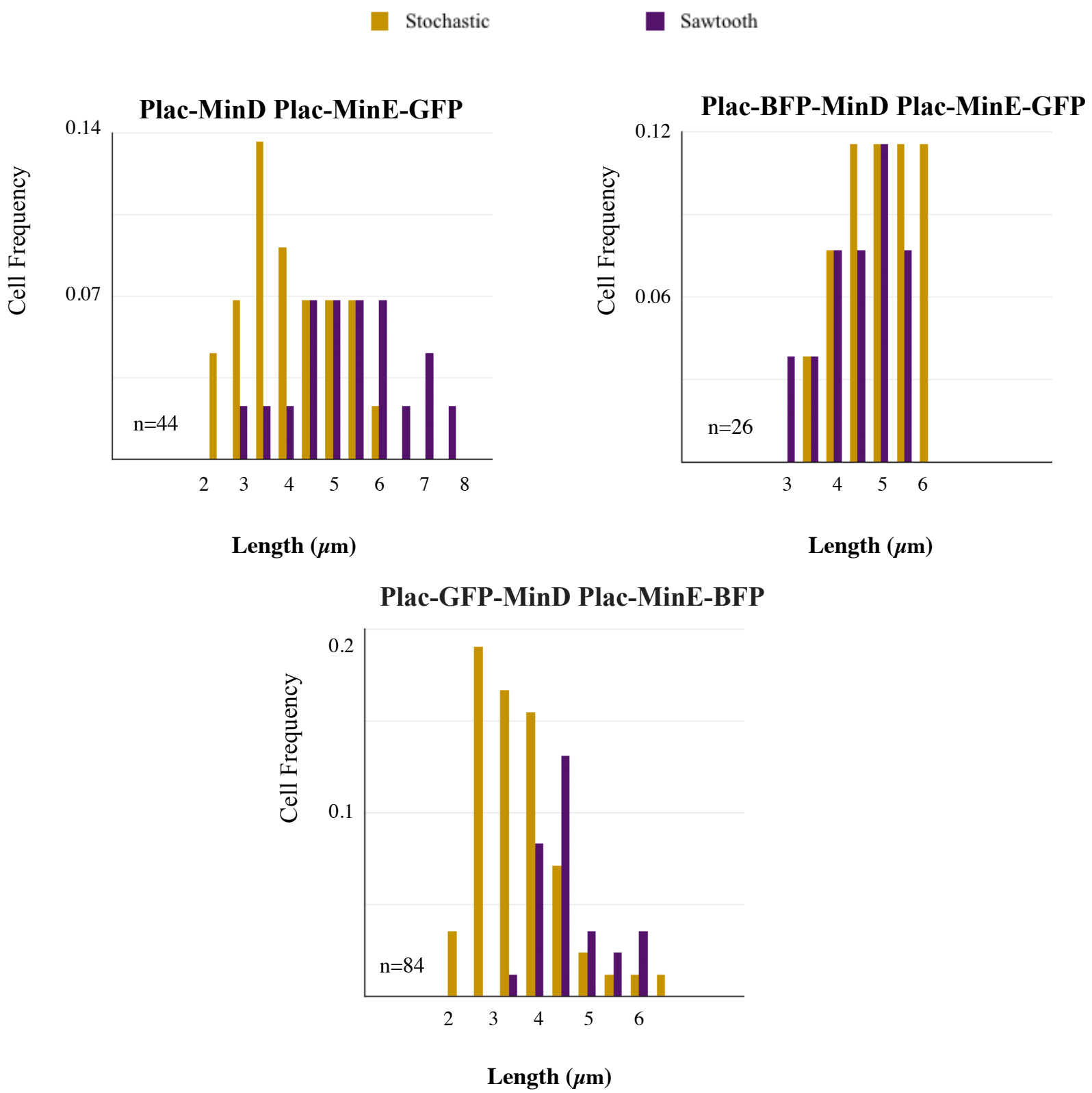



*Figure 9. A comparison of the plateau and sawtooth pattern types versus length and the frequency at which each occurs shows a normal distribution. This data is found from the first frame of a time series, meaning that all data points are independent of each other. This figure shows that there is almost no length in which we can predict a pattern, meaning that length alone is not an accurate determinant of protein oscillation pattern.*

**Figure 10. Insignificant Correlation Between Change in Length from Transition to Division and Change in Time Between Each Event**

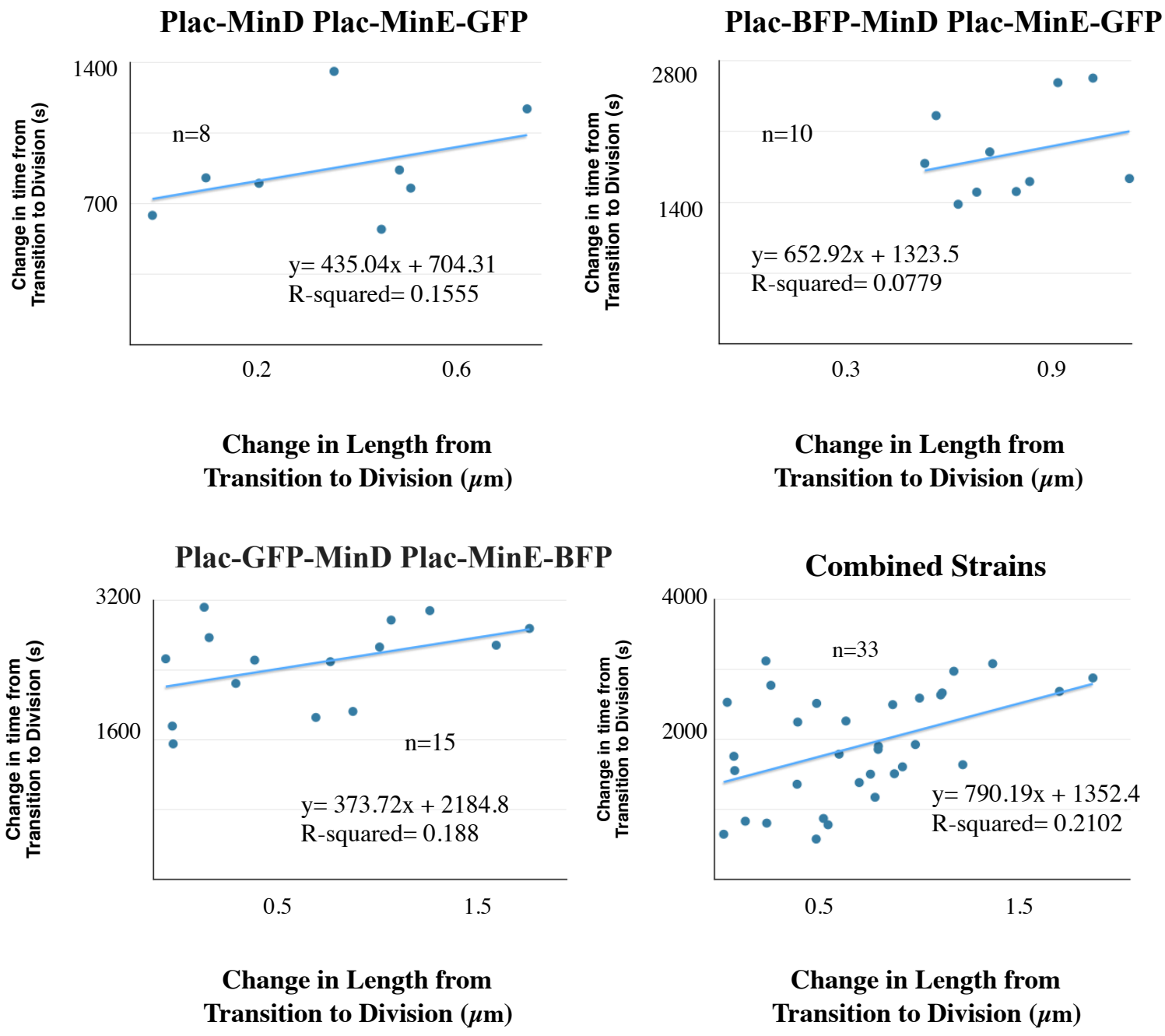
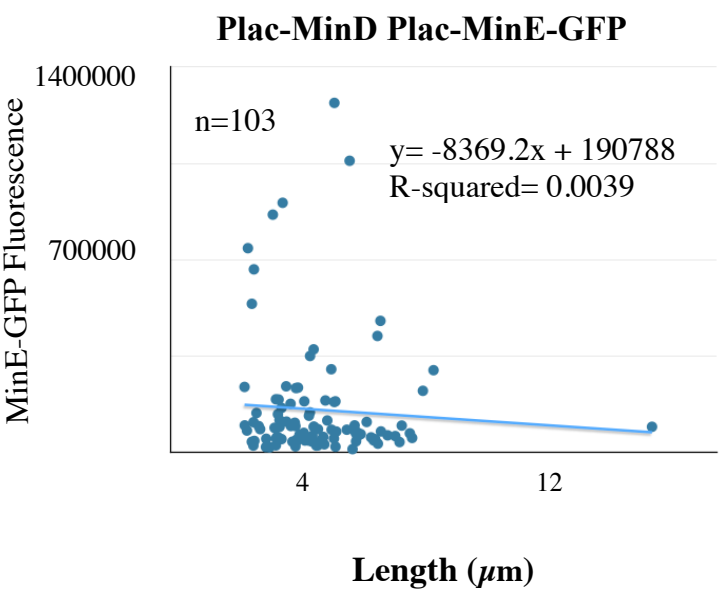
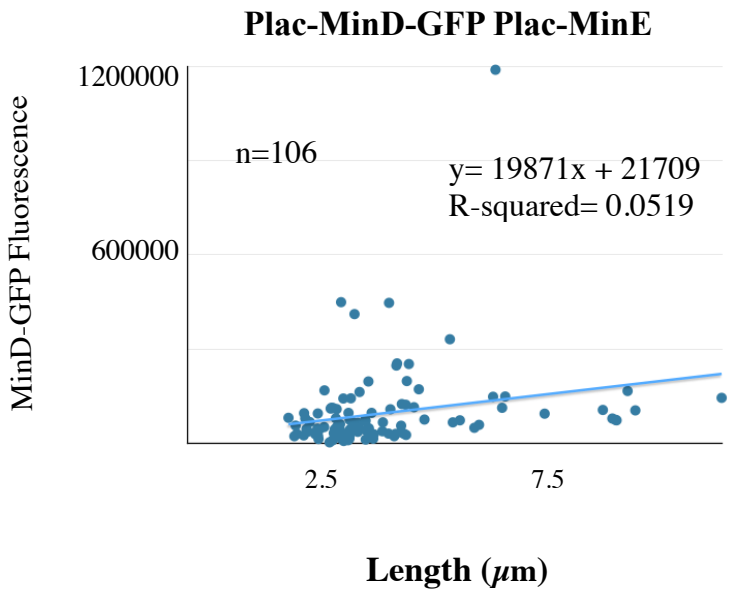
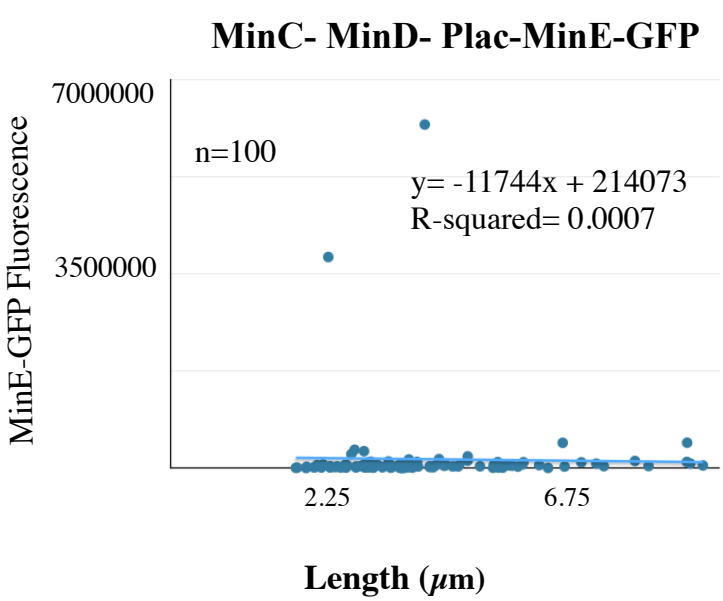


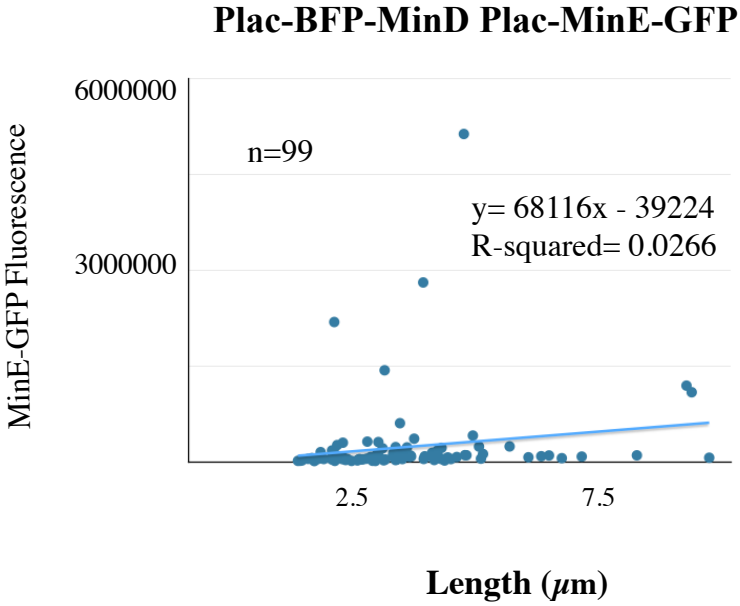

*Figure 10. Change in pole to pole cell length and the time it takes a cell to divide after it transitions from plateau oscillations to sawtooth oscillations are insignificantly correlated. This indicates that a cell can divide at any point in time following a transition.*

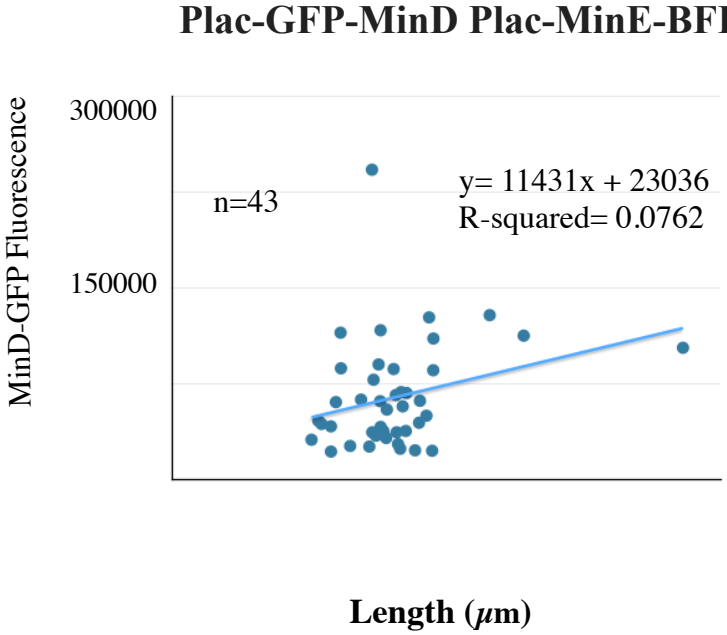
**Figure 11. No Correlation Between Min Concentrations and Length of Cell in All Strains Tested**



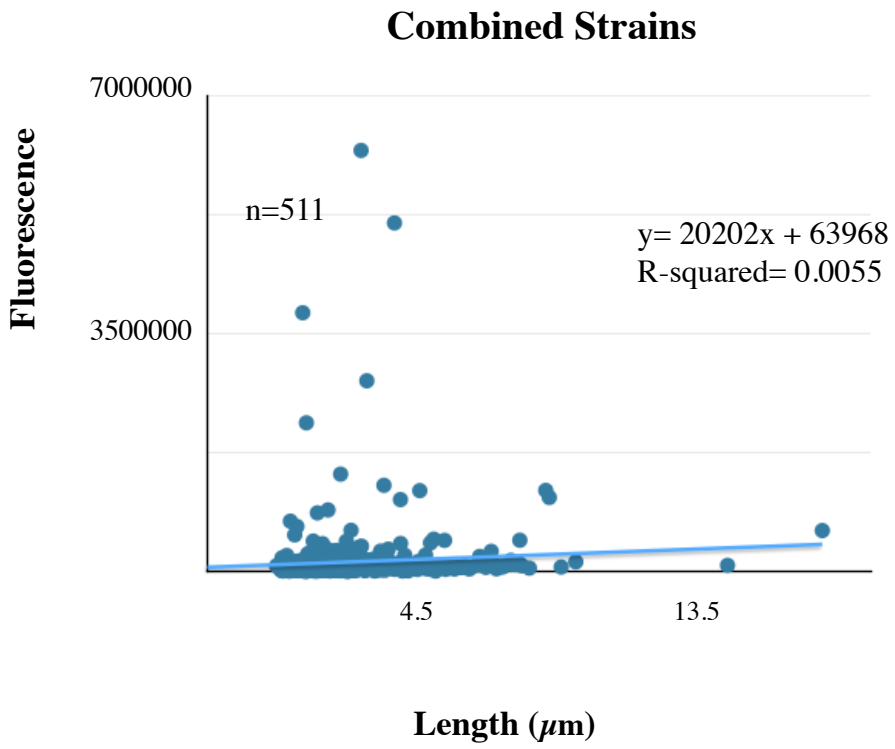



*Figure 11. All strains over expressing MinD and MinE show no correlation between length and fluorescence from single frame images. There is a wide variety of lengths that were tested, and concentration of Min proteins is not determined by any of them. This characteristic is valid when all data points are combined in one cohort, showing the lack of correlation is independent of genotype.*

**Evidence against length, time, and Min abundance as the regulator.**

Length is not a strict determiner of the pattern type, meaning the transition between oscillating patterns can happen at any length (Figure 9). The Gaussian fit statistics show significance for the normal distribution of length at transition and division. For all strains analyzed, the Sum of Squares Due to Error is near 0 indicating a small random error component, the R-Squared value is near 1 indicating a greater proportion of variance is accounted for, the Adjusted R-Squared value is near 1 indicating a great quality of fit for the curve, and the Root Mean Squared Error is near 0 indicating a fit that is more useful for predictions (Figure 7, Figure 8). Post-transition, there is no regulation on the amount of time that passes until the cell divides, which means that cell division is unpredictable (Figure 10). These results show that Min protein abundance is not the regulator of the events in the *E. coli* cell cycle, as the concentration of Min proteins is not correlated with length of the cell (Figure 11).

## Discussion

A major question in biology is: how are the events in a cell cycle regulated? The results found from this study help to determine how the events in an *E. coli* cell cycle are not regulated. Regulation is highly evident and finding what is not responsible for this control is certainly a step in finding out what is. In order for cells to proliferate properly, there needs to be dictation of the events in it's cycle and ruling out the cell length, time, and concentration of Min proteins as the internal cues open up the window for further investigation for discovering the actual regulator.

Studies prior to this have shown that protein content and the volume of *E.coli* are linearly related. One study by Ron Milo in 2013 used mass spectrometry and mathematical models to show that the amount of proteins increase per μm cubed increase. Another study presumed that rapidly growing cells have more DNA and protein increase in proportion to DNA. Using this, they found the ratio of proteins/DNA to be independent of growth rate, meaning protein abundance also increases with increasing cell growth (Dennis et al., 1974). Our results show that when the fluorescence of MinD and MinE were measured and graphed against the length of the same *E.coli* cell, there was no statistically significant correlation. This finding holds true for when a cell is first observed by taking a static image and analyzing the fluorescence in the absence of photobleaching. A possible explanation for the contrasting results is that the referenced experiments looked at proteins in general, whereas we focused strictly on the over expressed MinD and MinE proteins. Another reason for the contradiction in findings could be explained by the difference in the *E.coli* strains genes used in each experiment. A study done by Taniguchi et al. in 2010 found that some proteins are synthesized in a stochastic manner in *E.coli*, which results in seemingly random protein bursts. They found that there are fluctuations in mRNA and protein copy numbers within populations of genetically identical cells. Their findings support, and could explain, our result of Min proteins not scaling linearly with length. If bursts of proteins happen at different times, then some of our data points could have been in the middle of a larger protein burst than others when the data was recorded. The Min proteins themselves could be the regulators of these events simply by the nature of their protein quality, though we have shown that Min protein quantity is not.

For the strains used in this experiment, length does not dictate the amount of Min proteins within the cell at any event. However, length at transition and length at division show a

significant correlation, meaning something is tightly regulating those events in relation to each other and it is not the concentration of the Min proteins. If the concentration of Min proteins is not the determiner of transition and division, then what could be? It has been hypothesized that the length of the cell is the regulator for the events. In 2010, Fischer-Friedrich, et al. found Min proteins generally transitioned from plateau phase to sawtooth phase at a length between 2.5-3.0 μm. Our data shows that there is a normal distribution with a wide range of lengths associated with the transition phase (Figure 9). The Gaussian fit for the length at transition is statistically significant with minimal error. The mean cell frequency for our data is rather large, compared to 2.5-3.0 μm. In this same work done by Fischer-Friedrich, et al. in 2010, they found a critical length of 2.7 μm in which cells strictly exhibited a plateau oscillation pattern. Conversely, our data shows plateau oscillations into the 6 μm region (Figure 8). A cause for these discrepancies could be the difference in concentrations of IPTG used to induce the *lac* promoter or differences in the genomes of the strains used. We have also found that time is not a plausible candidate for the regulation. As time passes from the beginning of the cell cycle into transition of the oscillation patterns, the variability and time spent on each side of the cell decreases. However, there is no correlation between the amount of time that passes and the amount of growth in length a cell undergoes between transition and division. This means that division can happen at any time following a transition event, and a cue other than length, time, or Min protein concentration tells the cell when to divide.

Moving forward with this experiment, we plan to construct mathematical models to test hypotheses as to what could be the nature of the regulation behind the transition and division events in *E. coli.* We are also testing all strains with different concentrations of IPTG to see what happens when the operon is driven to different levels of expression of the transgenic MinD and MinE proteins.

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